

FORM PTO-1390 (REV. 10-2000)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 20330P
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/806087
INTERNATIONAL APPLICATION NO. PCT/US99/22634	INTERNATIONAL FILING DATE September 29, 1999	PRIORITY DATE CLAIMED October 2, 1998
TITLE OF INVENTION G PROTEIN-COUPLED RECEPTOR RESEMBLING THE THROMBIN RECEPTOR		
APPLICANT(S) FOR DO/EO/US QINGYUN LIU, TERRENCE P. McDONALD AND RUIPING WANG		
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input type="checkbox"/> This is an express request to begin national examination procedures [35 U.S.C. 371(f)] at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(i). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made and the US was elected by the expiration of the 19th month from the earliest claimed priority date (PCT Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed [35 U.S.C. 371(c)(2)]. <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> has been communicated by the International Bureau. c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed [35 U.S.C. 371(c)(2)]. 7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)]. <ol style="list-style-type: none"> a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)]. 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)]. 10. <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)]. <p>Items 11 to 16 below concern other document(s) or information included:</p> <ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 14. <input type="checkbox"/> A substitute specification. 15. <input type="checkbox"/> A change of power of attorney and/or address letter. 16. <input type="checkbox"/> Other items or information: 		

EXPRESS MAIL CERTIFICATE
 DATE OF DEPOSIT March 26, 2001
 EXPRESS MAIL NO. 92091363US
 I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS
 BEING DEPOSITED WITH THE UNITED STATES POSTAL
 SERVICE AS EXPRESS MAIL "POST OFFICE TO
 ADDRESSEE" BEFORE 5 P.M. ON THE ABOVE DATE IN
 AN ENVELOPE ADDRESSED TO ASSISTANT COMMISSIONER
 FOR PATENTS, WASHINGTON, D.C. 20230
 MAILED BY [Signature]
 DATE 3/26/01

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)		INTERNATIONAL APPLICATION NO		ATTORNEY'S DOCKET NUMBER	
09/806087		PCT/US99/22634		20330P	

17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE [37 CFR 1.492(a)(1)-(5)]: Neither international preliminary examination fee [37 CFR 1.482] nor international search fee [37 CFR 1.445(a)(2)] paid to USPTO and International Search Report not prepared by the EPO or JPO..... \$1,000.00 International preliminary examination fee [37 CFR 1.482] not paid to USPTO but International Search Report prepared by the EPO or JPO..... \$860.00 International preliminary examination fee [37 CFR 1.482] not paid to USPTO but international search fee [37 CFR 1.445(a)(2)] paid to USPTO. \$710.00 International preliminary examination fee paid to USPTO [37 CFR 1.482] but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... \$690.00 International preliminary examination fee paid to USPTO [37 CFR 1.482] and all claims satisfied provisions of PCT Article 33(1)-(4)..... \$100.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>	CALCULATIONS	PTO USE ONLY

Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date [37 CFR 1.492(e)].				\$0.00
---	--	--	--	--------

Claims	Number Filed	Number Extra	Rate	
Total Claims	17 - 20 =	0	X \$18.00	\$0.00
Independent Claims	11 - 3 =	8	X \$80.00	\$640.00
Multiple dependent claim(s) (if applicable)		0	+ \$270.00	\$0.00
TOTAL OF ABOVE CALCULATIONS =				\$740.00

<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				
SUBTOTAL =				\$740.00

Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date [37 CFR 1.492(f)].				+	\$0.00
TOTAL NATIONAL FEE =				\$740.00	

Fee for recording the enclosed assignment [37 CFR 1.21(h)]. The assignment must be accompanied by an appropriate cover sheet [37 CFR 3.28, 3.31]. \$40.00 per property.				+	
TOTAL FEES ENCLOSED =				\$740.00	
				Amount to be refunded	
				charged	

a. ☐ A check in the amount of \$ _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 13-2755 in the amount of \$740.00 to cover the above fees.
 A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
 overpayment to the Deposit Account No. 13-2755. A duplicate copy of this sheet is enclosed.


**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive
 [37 CFR 1.137(a) or (b)] must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

MERCK & CO., INC.
 Patent Department, RY60-30
 P.O. Box 2000
 126 East Lincoln Avenue
 Rahway, New Jersey 07065-0970

DATE: MARCH 26, 2001

PHONE #: (732) 594-6734


 SIGNATURE

 Joseph A. Coppola
 NAME

 38,413
 REGISTRATION NUMBER

09/05/00 087

TITLE OF THE INVENTION
G PROTEIN-COUPLED RECEPTOR RESEMBLING THE THROMBIN
RECEPTOR

5 CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

10

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

15

This invention relates to a novel human cDNA encoding HG52, a G protein-coupled receptor (GPCR) having homology to protease-activated receptors such as the thrombin receptors, the protein encoded by the cDNA, and methods of identifying selective agonists and antagonists of the protein encoded by the cDNA.

20

BACKGROUND OF THE INVENTION

G-protein coupled receptors (GPCRs) are a very large class of membrane receptors that relay information from the exterior to the interior of cells. GPCRs function by interacting with a class of heterotrimeric proteins known as G-proteins. Most GPCRs function by a similar mechanism. Upon the binding of
25 agonist, a GPCR catalyzes the dissociation of guanosine diphosphate (GDP) from the α subunit of G proteins. This allows for the binding of guanosine triphosphate (GTP) to the α subunit, resulting in the disassociation of the α subunit from the β and γ subunits. The freed α subunit then interacts with other cellular components, and in the process passes on the extracellular signal represented by the presence of the
30 agonist. Occasionally, it is the freed β and γ subunits which transduce the agonist signal.

GPCRs possess common structural characteristics. They have seven hydrophobic domains, about 20-30 amino acids long, linked by sequences of hydrophilic amino acids of varied length. These seven hydrophobic domains
35 intercalate into the plasma membrane, giving rise to a protein with seven

transmembrane domains, an extracellular amino terminus, and an intracellular carboxy terminus (Strader et al., 1994, *Ann. Rev. Biochem.* 63:101-132; Schertler et al., 1993, *Nature* 362:770-772; Dohlman et al., 1991, *Ann. Rev. Biochem.* 60:653-688).

5 GPCRs are expressed in a wide variety of tissue types and respond to a wide range of ligands, *e.g.*, protein hormones, biogenic amines, peptides, lipid derived messengers, etc. Given their wide range of expression and ligands, it is not surprising that GPCRs are involved in many pathological states. This has led to great interest in developing modulators of GPCR activity that can be used pharmacologically. For
10 example, Table 1 of Stadel et al., 1997, *Trends Pharmacol. Sci.* 18:430-437, lists 37 different marketed drugs that act upon GPCRs. Accordingly, there is a great need to understand GPCR function and to develop agents that can be used to modulate GPCR activity.

The thrombin receptors are GPCRS that are members of the seven
15 transmembrane domain receptor family. The first discovered thrombin receptor has a relatively long amino-terminal extracellular domain. This extracellular domain serves as a substrate for thrombin. Thrombin cleaves the extracellular domain at a site 41 amino acids from its N terminus, creating a new N terminus. This new N terminus activates the thrombin receptor by functioning as a tethered, intramolecular ligand
20 (Vu et al., 1991, *Cell* 64:1057-1068). The thrombin receptors may be responsible for mediating the effects of thrombin in platelets, endothelial cells, smooth muscle cells, fibroblasts, mesangial cells, and neural cells. For a review of the thrombin receptors, see Dennington & Berndt, 1994, *Clin. Exper. Pharmacol. Physiol.* 21:349-358.

The medical importance of the thrombin receptors is underscored by a
25 consideration of the variety of effects attributable to thrombin: conversion of fibrinogen to fibrin in plasma; activation of clotting factors V, VIII, XIII, and protein C; modulation of the procoagulant function of platelets and endothelial cells; and the stimulation of platelet activation (Davey & Luscher, 1967, *Nature* 216:857-858; Coughlin et al., 1992, *J. Clin. Invest.* 89:351-355); chemotaxis of monocytes (Bar-Shavit et al., 1983, *Science* 220:728-731) and lymphocytes (Bizios et al, 1985,
30 *Thrombosis Res.* 38:425-431); mitogenesis of lymphocytes and mesenchymal cells such as vascular smooth muscle cells, fibroblasts, and epithelial cells (Chen & Buchanan, 1975, *Proc. Natl Acad. Sci. USA* 72:131-135; Reddan et al., 1982, *Invest. Ophthal. Visual Sci.* 22:486-493). Modulation of the activity of the thrombin
35 receptors is one possibility for modulating the above-described effects of thrombin.

U.S. Patent No. 5,686,597 discloses a homologue of the human thrombin receptors.

SUMMARY OF THE INVENTION

The present invention is directed to a novel human cDNA that encodes a G-protein coupled receptor, HG52. The DNA encoding HG52 is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. Also provided is an HG52 protein encoded by the novel cDNA sequence. The HG52 protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:2. Methods of expressing HG52 in recombinant systems and of identifying agonists and antagonists of HG52 are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the complete cDNA sequence of HG52 (SEQ.ID.NO.:1).

Figure 2 show the complete amino acid sequence of HG52 (SEQ.ID.NO.:2).

Figure 3A-B shows the translation of the HG52 open reading frame. The nucleotide sequence shown is (SEQ.ID.NO.:1). The amino acid sequence shown is (SEQ.ID.NO.:2).

Figure 4 shows the results of a Northern blot of the expression of HG52 mRNA in various human tissues.

Figure 5 shows an alignment of the amino acid sequence of HG52 with the amino acid sequence of the human thrombin receptor 1 (SEQ.ID.NO.:3).

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this invention:

“Substantially free from other proteins” means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins.

Thus, an HG52 protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-HG52 proteins. Whether a given HG52 protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel

electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting.

“Substantially free from other nucleic acids” means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, an HG52 DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-HG52 nucleic acids. Whether a given HG52 DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

“Functional equivalent” means a receptor which does not have exactly the same amino acid sequence as naturally occurring HG52, due to alternative splicing, deletions, mutations, substitutions, or additions, but retains substantially the same biological activity as HG52. Such functional equivalents will have significant amino acid sequence identity with naturally occurring HG52. Genes and cDNA encoding such functional equivalents can be detected by reduced stringency hybridization with a DNA sequence encoding naturally occurring HG52. For the purposes of this invention, naturally occurring HG52 has the amino acid sequence shown as SEQ.ID.NO.:2 and is encoded by SEQ.ID.NO.:1. A nucleic acid encoding a functional equivalent has at least about 50% identity at the nucleotide level to SEQ.ID.NO.:1.

A polypeptide has “substantially the same biological activity” as HG52 if that polypeptide has a K_d for a ligand that is no more than 5-fold greater than the K_d of HG52 having SEQ.ID.NO.:2 for the same ligand.

A “conservative amino acid substitution” refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (*e.g.*, arginine for lysine; glutamic acid for aspartic acid).

By “isolated HG52 protein” is meant HG52 protein that has been isolated from a natural source. Use of the term “isolated” indicates that HG52 protein has been removed from its normal cellular environment. Thus, an isolated HG52

protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated HG52 protein is the only protein present, but instead means that an isolated HG52 protein is at least 95% free of non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the HG52 protein. Thus, an HG52 protein that is expressed in bacteria or even in eukaryotic cells which do not naturally (*i.e.*, without human intervention) express it through recombinant means is an "isolated HG52 protein."

One aspect of this invention is the identification and cloning of a novel G protein-coupled receptor (GPCR), substantially free from other proteins, designated as HG52.

Another aspect of this invention are nucleic acids which encode the HG52 G protein-coupled receptor. These nucleic acids are substantially free from other nucleic acids.

The present invention provides a cDNA molecule substantially free from other nucleic acids having the nucleotide sequence shown in Figure 1 as SEQ.ID.NO.:1. SEQ.ID.NO.:1 contains an open reading frame (positions 23-1,099 of SEQ.ID.NO.:1) encoding a protein of 359 amino acids (see Figure 3A-B). Thus, the present invention also provides a DNA molecule substantially free from other nucleic acids comprising the nucleotide sequence of positions 23-1,099 of SEQ.ID.NO.:1. The present invention also provides recombinant DNA molecules comprising the nucleotide sequence of positions 23-1,099 of SEQ.ID.NO.:1.

Based on its predicted amino acid sequence, the HG52 protein most likely represents a novel G-protein coupled receptor (GPCR) of the rhodopsin family since the HG52 protein contains many of the characteristic features of GPCRs of the rhodopsin family, *viz.*

- (a) seven transmembrane domains;
- (b) homology with members of the rhodopsin family of GPCRs;
- (c) signature motifs of GPCRs in the rhodopsin family.

Northern blot analysis (Figure 4) showed that HG52 RNA is widely expressed in humans as a transcript of about 4.5 kb, especially in cells of the immune system (peripheral blood lymphocytes (PBLs), spleen, bone marrow, lymph nodes). This argues for a role for HG52 in immune system function.

The novel DNA sequences of the present invention encoding HG52, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to

which HG52 is not naturally linked, to form "recombinant DNA molecules" containing HG52. The novel DNA sequences of the present invention can be inserted into vectors in order to direct recombinant expression of HG52. Such vectors may be comprised of DNA or RNA; for most purposes DNA vectors are preferred. Typical
5 vectors include plasmids, modified viruses, bacteriophage, cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA that can encode HG52. One skilled in the art can readily determine an appropriate vector for a particular use.

Included in the present invention are cDNA sequences that hybridize to SEQ.ID.NO.:1 under stringent conditions. By way of example, and not limitation,
10 a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10⁶ cpm of ³²P-labeled probe.
15 Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

Other procedures using conditions of high stringency would include either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50%
20 formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, e.g., Sambrook, Fritsch, and Maniatis, 1989,
25 Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the
30 construction of synthetic DNA that encodes the HG52 protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ.ID.NO.:1, but still encodes the same HG52 protein as SEQ.ID.NO.:1. Such synthetic DNAs are intended to be within the scope of the present invention. If it is desired to express such synthetic DNAs in a particular host cell or organism, the
35 codon usage of such synthetic DNAs can be adjusted to reflect the codon usage of

that particular host, thus leading to higher levels of expression of HG52 protein in the host.

Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding HG52. Such recombinant host cells can be cultured under suitable conditions to produce HG52. An expression vector containing DNA encoding HG52 can be used for expression of HG52 in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey, and rodent origin, and insect cells including but not limited to, *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable for recombinant expression of HG52 and which are commercially available, include but are not limited to, L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

Human embryonic kidney (HEK 293) cells and Chinese hamster ovary (CHO) cells are particularly suitable for expression of the HG52 protein because these cells express a large number of G-proteins. Thus, it is likely that at least one of these G-proteins will be able to functionally couple the signal generated by interaction of HG52 and its ligands, thus transmitting this signal to downstream effectors, eventually resulting in a measurable change in some assayable component, e.g., cAMP level, expression of a reporter gene, hydrolysis of inositol lipids, or intracellular Ca²⁺ levels.

Other cells that are particularly suitable for expression of the HG52 protein are immortalized melanophore pigment cells from *Xenopus laevis*. Such melanophore pigment cells can be used for functional assays using recombinant expression of HG52 in a manner similar to the use of such melanophore pigment cells for the functional assay of other recombinant GPCRs (Graminski et al., 1993, J. Biol. Chem. 268:5957-5964; Lerner, 1994, Trends Neurosci. 17:142-146; Potenza & Lerner, 1992, Pigment Cell Res. 5:372-378).

A variety of mammalian expression vectors can be used to express recombinant HG52 in mammalian and other cells. Commercially available

mammalian expression vectors which are suitable include, but are not limited to, pCR2.1 (Invitrogen), pMC1neo (Stratagene), pSG5 (Stratagene), pcDNA1 and pcDNA1amp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). For expression in non-mammalian cells, various suitable expression vectors are known in the art. The choice of vector will depend upon cell type used, level of expression desired, and the like. Following expression in recombinant cells, HG52 can be purified by conventional techniques to a level that is substantially free from other proteins.

The present invention includes HG52 protein substantially free from other proteins. The amino acid sequence of the full-length HG52 protein is shown in Figure 2 as SEQ.ID.NO.:2. Thus, the present invention includes HG52 protein substantially free from other proteins having the amino acid sequence of SEQ.ID.NO.:2.

As with many receptor proteins, it is possible to modify many of the amino acids of HG52, particularly those which are not found in the ligand binding domain, and still retain substantially the same biological activity as the original receptor. Thus this invention includes modified HG52 polypeptides which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as HG52. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, e.g., Molecular Biology of the Gene, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, Science 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO.:2 wherein the polypeptides still retain substantially the same biological activity as HG52. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO.:2 wherein the polypeptides still retain substantially the same biological activity as HG52. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in the ligand-binding domain of HG52.

When deciding which amino acid residues of HG52 may be substituted to produce polypeptides that are functional equivalents of HG52, one skilled in the art would be guided by a comparison of the amino acid sequence of HG52 with the amino acid sequences of related proteins, *e.g.*, the human thrombin receptors (see Vu et al., 1991, Cell 64:1057-1068; Ishihara et al., 1997, Nature 386:502-506; and Figure 5 of the present application). Such a comparison would allow one skilled in the art to minimize the number of amino acid substitutions made in regions that are highly conserved between HG52 and the related protein. Accordingly, the present invention includes embodiments where the substitutions are conservative and do not occur in positions where HG52 and the human thrombin receptor 1 share the same amino acid (see Figure 5).

One skilled in the art would also recognize that polypeptides that are functional equivalents of HG52 and have changes from the HG52 amino acid sequence that are small deletions or insertions of amino acids could also be produced by following the same guidelines, *i.e.*, minimizing the differences in amino acid sequence between HG52 and related proteins. Small deletions or insertions are generally in the range of about 1 to 5 amino acids. The effect of such small deletions or insertions on the biological activity of the modified HG52 polypeptide can easily be assayed by producing the polypeptide synthetically or by making the required changes in DNA encoding HG52 and then expressing the DNA recombinantly and assaying the protein produced by such recombinant expression.

The present invention also includes C-terminal truncated forms of HG52, particularly those which encompass the extracellular portion of the receptor, but lack the intracellular signaling portion of the receptor. Such truncated receptors are useful in various binding assays described herein, for crystallization studies, and for structure-activity-relationship studies.

It has been found that, in some cases, membrane spanning regions of receptor proteins can be used to inhibit receptor function (Ng *et al.*, 1996, Biochem. Biophys. Res. Comm. 227:200-204; Hebert *et al.*, 1996, J. Biol. Chem. 271, 16384-16392; Lofts *et al.*, Oncogene 8:2813-2820). Accordingly, the present invention provides peptides derived from the seven membrane spanning regions of HG52 and their use to inhibit HG52 function. Such peptides can include whole or parts of the receptor membrane spanning domains. Among such peptides are peptides containing amino acid residues 31-49, 61-82, and 98-119 of SEQ.ID.NO.:2.

The present invention also includes chimeric HG52 proteins. Chimeric HG52 proteins consist of a contiguous polypeptide sequence of HG52 fused in frame to a polypeptide sequence of a non-HG52 protein. For example, the N-terminal domain and seven transmembrane spanning domains of HG52 fused at the C-terminus in frame to a G protein would be a chimeric HG52 protein.

The present invention also includes HG52 proteins that are in the form of multimeric structures, *e.g.*, dimers. Such multimers of other G-protein coupled receptors are known (Hebert *et al.*, 1996, J. Biol. Chem. 271, 16384-16392; Ng *et al.*, 1996, Biochem. Biophys. Res. Comm. 227, 200-204; Romano *et al.*, 1996, J. Biol. Chem. 271, 28612-28616).

The present invention also includes isolated forms of HG52 proteins.

The present invention includes methods of identifying compounds that specifically bind to HG52 protein, as well as compounds identified by such methods. The specificity of binding of compounds having affinity for HG52 is shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from those cells. Expression of the cloned receptor and screening for compounds that bind to HG52 or that inhibit the binding of a known, radiolabeled ligand of HG52 to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of compounds with high affinity for HG52. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of HG52 and may be peptides, proteins, or non-proteinaceous organic molecules.

Therefore, the present invention includes assays by which HG52 agonists and antagonists may be identified. Methods for identifying agonists and antagonists of other receptors are well known in the art and can be adapted to identify agonists and antagonists of HG52. Accordingly, the present invention includes a method for determining whether a substance is a potential agonist or antagonist of HG52 that comprises:

- (a) transfecting cells with an expression vector encoding HG52;
- (b) allowing the transfected cells to grow for a time sufficient to allow HG52 to be expressed;
- (c) exposing the cells to a labeled ligand of HG52 in the presence and in the absence of the substance;

(d) measuring the binding of the labeled ligand to HG52; where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of HG52.

5 The conditions under which step (c) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

10 The present invention also includes a method for determining whether a substance is capable of binding to HG52, *i.e.*, whether the substance is a potential agonist or an antagonist of HG52, where the method comprises:

 (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG52 in the cells;

15 (b) exposing the test cells to the substance;

 (c) measuring the amount of binding of the substance to HG52;

 (d) comparing the amount of binding of the substance to HG52 in the test cells with the amount of binding of the substance to control cells that have not been transfected with HG52;

20 wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to HG52. Determining whether the substance is actually an agonist or antagonist can then be accomplished by the use of functional assays such as, *e.g.*, the assay involving the use of promiscuous G-proteins described below.

25 The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

30 In a particular embodiment of the above-described methods, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61),

3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

The assays described above can be carried out with cells that have been transiently or stably transfected with HG52. Transfection is meant to include any method known in the art for introducing HG52 into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing HG52, and electroporation.

Where binding of the substance or agonist to HG52 is measured, such binding can be measured by employing a labeled substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, *e.g.*, radioactively, fluorescently, enzymatically.

In particular embodiments of the above-described methods, HG52 has an amino acid sequence of SEQ.ID.NO.:2.

The above-described methods can be modified in that, rather than exposing the test cells to the substance, membranes can be prepared from the test cells and those membranes can be exposed to the substance. Such a modification utilizing membranes rather than cells is well known in the art and is described in, *e.g.*, Hess *et al.*, 1992, Biochem. Biophys. Res. Comm. 184:260-268.

Accordingly, the present invention provides a method for determining whether a substance is capable of binding to HG52 comprising:

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG52 in the cells;
- (b) preparing membranes containing HG52 from the test cells and exposing the membranes to a ligand of HG52 under conditions such that the ligand binds to the HG52 in the membranes;
- (c) subsequently or concurrently to step (b), exposing the membranes from the test cells to a substance;
- (d) measuring the amount of binding of the ligand to the HG52 in the membranes in the presence and the absence of the substance;
- (e) comparing the amount of binding of the ligand to HG52 in the membranes in the presence and the absence of the substance where a decrease in the amount of binding of the ligand to HG52 in the membranes in the presence of the substance indicates that the substance is capable of binding to HG52;

where HG52 has an amino acid sequence of SEQ.ID.NO.:2.

The present invention provides a method for determining whether a substance is capable of binding to HG52 comprising:

- (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG52 in the cells;
- 5 (b) preparing membranes containing HG52 from the test cells and exposing the membranes from the test cells to the substance;
- (c) measuring the amount of binding of the substance to the HG52 in the membranes from the test cells;
- (d) comparing the amount of binding of the substance to HG52 in
10 the membranes from the test cells with the amount of binding of the substance to membranes from control cells that have not been transfected with HG52;
- where HG52 has an amino acid sequence of SEQ.ID.NO.:2;
- where if the amount of binding of the substance to HG52 in the
15 membranes from the test cells is greater than the amount of binding of the substance to the membranes from the control cells, then the substance is capable of binding to HG52.

As a further modification of the above-described methods, RNA encoding HG52 can be prepared as, *e.g.*, by *in vitro* transcription using a plasmid containing HG52 under the control of a bacteriophage T7 promoter, and the RNA can
20 be microinjected into *Xenopus* oocytes in order to cause the expression of HG52 in the oocytes. Substances are then tested for binding to the HG52 expressed in the oocytes. Alternatively, rather than detecting binding, the effect of the substances on the electrophysiological properties of the oocytes can be determined.

The present invention includes assays by which HG52 agonists and
25 antagonists may be identified by their ability to stimulate or antagonize a functional response mediated by HG52. HG52 belongs to the class of proteins known as G-protein coupled receptors (GPCRs). GPCRs transmit signals across cell membranes upon the binding of ligand. The ligand-bound GPCR interacts with a heterotrimeric G-protein, causing the α subunit of the G-protein to disassociate from the β and
30 γ subunits. The α subunit can then go on to activate a variety of second messenger systems.

Generally, a particular GPCR is only coupled to a particular type of G-protein. Thus, to observe a functional response from the GPCR, it is necessary to
35 ensure that the proper G-protein is present in the system containing the GPCR. It has been found, however, that there are certain G-proteins that are "promiscuous." These

promiscuous G-proteins will couple to, and thus transduce a functional signal from, virtually any GPCR. See Offermanns & Simon, 1995, J. Biol. Chem. 270:15175-15180 (Offermanns). Offermanns described a system in which cells are transfected with expression vectors that result in the expression of one of a large number of GPCRs as well as the expression of one of the promiscuous G-proteins $G\alpha 15$ or $G\alpha 16$. Upon the addition of an agonist of the GPCR to the transfected cells, the GPCR was activated and was able, via $G\alpha 15$ or $G\alpha 16$, to activate the β isoform of phospholipase C, leading to an increase in inositol phosphate levels in the cells.

Therefore, by making use of these promiscuous G-proteins as in Offermanns, it is possible to set up functional assays for HG52, even in the absence of knowledge of the G-protein with which HG52 is coupled *in vivo*. One possibility is to create a fusion or chimeric protein composed of the extracellular and membrane spanning portion of HG52 fused to a promiscuous G-protein. Such a fusion protein would be expected to transduce a signal following binding of ligand to the HG52 portion of the fusion protein. Accordingly, the present invention provides a method of identifying antagonists of HG52 comprising:

- (a) providing cells that expresses a chimeric HG52 protein fused at its C-terminus to a promiscuous G-protein;
- (b) exposing the cells to an agonist of HG52;
- (c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG52;
- (d) measuring the level of inositol phosphates in the cells; where a decrease in the level of inositol phosphates in the cells in the presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an antagonist of HG52.

Another possibility for utilizing promiscuous G-proteins in connection with HG52 includes a method of identifying agonists of HG52 comprising:

- (a) providing cells that expresses both HG52 and a promiscuous G-protein;
- (b) exposing the cells to a substance that is a suspected agonist of HG52;
- (c) measuring the level of inositol phosphates in the cells; where an increase in the level of inositol phosphates in the cells as compared to the level of inositol phosphates in the cells in the absence of the suspected agonist indicates that the substance is an agonist of HG52.

Levels of inositol phosphates can be measured by monitoring calcium mobilization. Intracellular calcium mobilization is typically assayed in whole cells under a microscope using fluorescent dyes or in cell suspensions via luminescence using the aequorin assay.

5 In a particular embodiment of the above-described methods, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61),
10 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) or MRC-5 (ATCC CCL 171).

In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of HG52 and the promiscuous G-protein in the cells.

15 The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

20 In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of Gα15 or Gα16. Expression vectors containing Gα15 or Gα16 are known in the art. See, *e.g.*, Offermanns; Buhl *et al.*, 1993, FEBS Lett. 323:132-134; Amatruda *et al.*, 1993, J. Biol. Chem. 268:10139-10144.

25 The above-described assay can be easily modified to form a method to identify antagonists of HG52. Such a method is also part of the present invention and comprises:

- (a) providing cells that expresses both HG52 and a promiscuous G-protein;
- 30 (b) exposing the cells to a substance that is an agonist of HG52;
- (c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG52;
- (d) measuring the level of inositol phosphates in the cells;
- 35 where a decrease in the level of inositol phosphates in the cells in the presence of the suspected antagonist as compared to the level of inositol phosphates

in the cells in the absence of the suspected antagonist indicates that the substance is an antagonist of HG52.

In a particular embodiment of the above-described method, the cells are eukaryotic cells. In another embodiment, the cells are mammalian cells. In other embodiments, the cells are L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The conditions under which steps (b) and (c) of the method are practiced are conditions that are typically used in the art for the study of protein-ligand interactions: *e.g.*, physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C.

In a particular embodiment of the above-described method, the cells are transfected with expression vectors that direct the expression of HG52 and the promiscuous G-protein in the cells.

In a particular embodiment of the above-described method, the promiscuous G-protein is selected from the group consisting of Gα15 or Gα16.

In particular embodiments of the above-described methods, HG52 has an amino acid sequence of SEQ.ID.NO.:2.

While the above-described methods are explicitly directed to testing whether "a" substance is an agonist or antagonist of HG52, it will be clear to one skilled in the art that such methods can be adapted to test collections of substances, *e.g.*, combinatorial libraries, to determine whether any members of such collections are activators or inhibitors of HG52. Accordingly, the use of collections of substances, or individual members of such collections, as the substance in the above-described methods is within the scope of the present invention.

Agonists and antagonists of HG52 that are identified by the above-described methods should have utility in the treatment of diseases that involve the inappropriate expression of HG52. In particular, given the expression pattern of HG52 (see Figure 4), such agonists and antagonists should have utility in the treatment of various immune system disorders or disorders involving inflammation, *e.g.*, arthritis, asthma, lupus. Such agonists and antagonists should also be useful in treating various infectious diseases.

Given the resemblance between HG52 and the thrombin receptors, it is anticipated that agonists and antagonists of HG52 will have pharmacological activity and thus be useful in a similar manner to the manner in which agonists and antagonists of the thrombin receptors are useful, *viz.*, as modulators of the effects of thrombin. Accordingly, it is believed that agonists and antagonists of HG52 will have utility in modulating: the conversion of fibrinogen to fibrin in plasma; the activation of clotting factors V, VIII, XIII, and protein C; the procoagulant function of platelets and endothelial cells; the stimulation of platelet activation; chemotaxis of monocytes and lymphocytes; mitogenesis of lymphocytes and mesenchymal cells such as vascular smooth muscle cells, fibroblasts, and epithelial cells.

The present invention includes pharmaceutical compositions comprising agonists and antagonists of HG52. The agonists and antagonists are generally combined with pharmaceutically acceptable carriers to form pharmaceutical compositions. Examples of such carriers and methods of formulation of pharmaceutical compositions containing agonists and antagonists and carriers can be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain a therapeutically effective amount of the agonists and antagonists.

Therapeutic or prophylactic compositions are administered to an individual in amounts sufficient to treat or prevent conditions where HG52 activity is abnormal. The effective amount can vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration. The appropriate amount can be determined by a skilled physician.

Compositions can be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents can be desirable.

The compositions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compositions can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they can also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

Advantageously, compositions can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or

four times daily. Furthermore, compositions can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage

- 5 administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

- The dosage regimen utilizing the compositions is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route
10 of administration; the renal, hepatic and cardiovascular function of the patient; and the particular composition thereof employed. A physician of ordinary skill can readily determine and prescribe the effective amount of the composition required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of composition within the range that yields efficacy without
15 toxicity requires a regimen based on the kinetics of the composition's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a composition.

- The present invention also includes methods of expressing HG52 in recombinant systems and then utilizing the recombinantly expressed HG52 for
20 counter-screening. When screening compounds in order to identify potential pharmaceuticals that specifically interact with a target receptor, it is necessary to ensure that the compounds identified are as specific as possible for the target receptor. To do this, it is necessary to screen the compounds against as wide an array as possible of receptors that are similar to the target receptor. Thus, in order to find
25 compounds that are potential pharmaceuticals that interact with receptor A, it is necessary not only to ensure that the compounds interact with receptor A (the "plus target") and produce the desired pharmacological effect through receptor A, it is also necessary to determine that the compounds do not interact with receptors B, C, D, etc. (the "minus targets"). In general, as part of a screening program, it is important to
30 have as many minus targets as possible (see Hodgson, 1992, Bio/Technology 10:973-980, at 980). Therefore, HG52 proteins and DNA encoding HG52 proteins have utility in counter-screens. That is, they can be used as "minus targets" in counter-screens in connection with screening projects designed to identify compounds that specifically interact with other G-protein coupled receptors.

The DNA of the present invention, or hybridization probes based upon the DNA, can be used in chromosomal mapping studies in order to identify the chromosomal locations of the HG52 gene or of genes encoding proteins related to HG52. Such mapping studies can be carried out using well-known genetic and/or chromosomal mapping techniques such as, *e.g.*, linkage analysis with respect to known chromosomal markers or *in situ* hybridization. See, *e.g.*, Verma et al., 1988, Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York, NY. After identifying the chromosomal location of the HG52 gene or genes encoding proteins related to HG52, this information can be compared with the locations of known disease-causing genes contained in genetic map data (such as the data found in the Genome Issue of Science (1994, 265:1981-2144). In this way, one can correlate the chromosomal location of the HG52 gene or of genes encoding proteins related to HG52 with the locations of known disease-causing genes and thus help to limit the region of DNA containing such disease-causing genes. This will simplify the process of cloning such disease-causing genes. Also, once linkage between the chromosomal location of the HG52 gene or of genes encoding proteins related to HG52 and the location of a known disease-causing gene is established, that linkage can be used diagnostically to identify restriction fragment length polymorphisms (RFLPs) in the vicinity of the HG52 gene or of genes encoding proteins related to HG52. Such RFLPs will be associated with the disease-causing gene and thus can be used to identify individuals carrying the disease-causing gene.

For such chromosomal mapping studies as described herein, it may be advantageous to use, in addition to the DNA of the present invention, the reverse complement of the DNA of the present invention or RNA corresponding to the DNA of the present invention.

The present invention also includes antibodies to the HG52 protein. Such antibodies may be polyclonal antibodies or monoclonal antibodies and are useful in treating disorders of the immune system that involve the inappropriate expression or activity of the HG52 protein. The antibodies of the present invention are raised against the entire HG52 protein or against suitable antigenic fragments of the protein that are coupled to suitable carriers, *e.g.*, serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art. See, *e.g.*, Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer Applications in the Biosciences) 4:181-186.

For the production of polyclonal antibodies, HG52 protein or an antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an appropriate non-human host animal such as, *e.g.*, rabbits, sheep, goats, rats, mice. The animals are bled periodically and sera obtained are tested for the presence of antibodies to the injected antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, HG52 protein or an antigenic fragment, coupled to a suitable carrier, is injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, *Nature* 256:495-497. For a fuller description of the production of monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

Gene therapy may be used to introduce HG52 polypeptides into the cells of target organs. Nucleotides encoding HG52 polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, and polio virus based vectors. Alternatively, nucleotides encoding HG52 polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with HG52 polypeptides will be particularly useful for the treatment of diseases where it is beneficial to elevate HG52 activity.

A cDNA fragment encoding full-length HG20 can be isolated from an appropriate human cDNA library by using the polymerase chain reaction (PCR) employing suitable primer pairs. Such primer pairs can be selected based upon the cDNA sequence for HG20 shown in Figure 1 as SEQ.ID.NO.:1. Suitable primer pairs would be, *e.g.*:

ACCCTCCAGGATGCAGG (SEQ.ID.NO.:4) and

ACTCAGAACACACTCTCC (SEQ.ID.NO.:5).

The above primers are meant to be illustrative. One skilled in the art would recognize that a variety of other suitable primers can be designed. For

example, the primers HG52.NotI (SEQ.ID.NO.:6) and HG52.KpnI (SEQ.ID.NO.:7), see Example 1, could be used.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM MgCl₂, 200 μM for each dNTP, 50 mM KCl, 0.2 μM for each primer, 10 ng of DNA template, 0.05 units/μl of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer, A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990, Academic Press.

A suitable cDNA library from which a clone encoding HG52 can be isolated would be a human cDNA library made from RNA from peripheral blood lymphocytes, spleen, lymph nodes, or bone marrow. Such libraries can be prepared by methods well-known in the art. Alternatively, several commercially available libraries would be suitable, e.g., the human leukocyte 5'-stretch plus cDNA library and human spleen 5'-stretch plus cDNA library of Clontech Laboratories, Inc. (Palo Alto, CA, USA). The primary clones of such libraries can be subdivided into pools with each pool containing approximately 20,000 clones and each pool can be amplified separately.

By this method, a cDNA fragment encoding an open reading frame of 359 amino acids (SEQ.ID.NO.:2) can be obtained. This cDNA fragment can be cloned into a suitable cloning vector or expression vector. For example, the fragment can be cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, Ca). HG52 protein can then be produced by transferring an expression vector encoding HG52 or portions thereof into a suitable host cell and growing the host cell under appropriate conditions. HG52 protein can then be isolated by methods well known in the art.

As an alternative to the above-described PCR method, a cDNA clone encoding HG52 can be isolated from a cDNA library using as a probe oligonucleotides specific for HG52 and methods well known in the art for screening cDNA libraries with oligonucleotide probes. Such methods are described in, e.g., Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring

Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K., Vol. I, II.

Oligonucleotides that are specific for HG52 and that can be used to screen cDNA libraries can be readily designed based upon the cDNA sequence of HG52 shown in Figure 1 as SEQ.ID.NO.:1 and can be synthesized by methods well-known in the art.

- 5 Genomic clones containing the HG52 gene can be obtained from commercially available human PAC or BAC libraries, *e.g.*, from Research Genetics, Huntsville, AL. Alternatively, one may prepare genomic libraries, especially in P1 artificial chromosome vectors, from which genomic clones containing the HG52 can be isolated, using probes based upon the HG52 nucleotide sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou *et al.*, 1994, *Nature Genet.* 6:84-89).
- 10

- The following non-limiting examples are presented to better illustrate the invention.
- 15

EXAMPLE 1

Cloning and sequencing of HG52

- An EST (GenBank accession number aa804531) was identified as a putative G-protein coupled receptor. Primers HG52.F91 and HG52.R242 were designed from the EST sequence and used to screen a superpool plasmid library derived from tissues of fetal brain, prostate, placenta, and testis. RCCA was then performed to isolated the full-length coding sequence of HG52 from individual library pools. In the primary reaction, primer HG52.F91 was paired with either PBS.873F or PBS.543R and HG52.R310 was paired with either PBS.873F or PBS.543R. In the secondary reaction, primer HG52.F165 was paired with PBS.838F or PBS.578R and HG52.R242 was paired with PBS.838F or PBS.578R. PCR products were sequenced and assembled with the aa804531 EST as a guide. Primers HG52.506F and HG52.603R were designed from new sequence and used to identify new pools in the superpool and tissue specific plasmid libraries. RCCA was carried out on these new pools with the following primer combinations: HG52.506F with PBS.543R or PBS.873F and HG52.623R with PBS.543R or PBS.873F. PCR products were sequenced and assembled. Nested RCCA was then done to identify
- 20
- 25
- 30

- more specific and longer PCR products. In the primary reaction, HG52.357F was paired with either PBS.543R or PBS.873F and HG52.623R was paired with PBS.543R or PBS.873F. In the secondary reaction, HG52.F506 was paired with either PBS.578R or PBS.838F and HG52.526R was paired with either PBS.578R or PBS.838F. PCR products were purified, sequenced, and assembled into a contig. In order to identify new 5' transcripts, a human leukocyte phage library was scanned with primers HG52.357F and HG52.623R. Nested RCCA was then done on the positive wells using the following primer combinations: In the primary reaction, HG52.357F was paired with either lambda gt10 3' or lambda gt10 5' and HG52.623R with lambda gt10 3' or lambda gt10 5'. In the secondary reaction, HG52.506F was paired with either lambda gt10 3' or lambda gt10 5' and HG52.526R was paired with lambda gt10 3' or lambda gt10 5'. PCR products were then purified and sequenced and assembled into the contig. From these PCR extensions and sequencing, an open reading frame of 1,080bp encoding a polypeptide sequence of 359 amino acids was obtained. The full length cDNA encoding HG52 was then amplified using primers HG52.NotI and HG52.KpnI and subcloned into the expression vector pcDNA3.1(+). The sequences of the primers used to sequence and clone HG52 are listed below:

PRIMERS

- | | | |
|----|-----------|--|
| 20 | HG52.NotI | GCGGCCGCTCCCTGAACCTCTGGCACC (SEQ.ID.NO.:6) |
| | HG52.KpnI | GCGGTACCATGCAGGTCCCGAAC (SEQ.ID.NO.:7) |
| | HG52.165F | GCTACTTCTGCCGCTGCTTC (SEQ.ID.NO.:8) |
| | HG52.310R | TGCTGACCGCCCCACCA (SEQ.ID.NO.:9) |
| 25 | HG52.506F | ACCATTGCAACCGCCACCA (SEQ.ID.NO.:10) |
| | HG52.623R | GGTACAGGACCCCGAGGAAGC (SEQ.ID.NO.:11) |
| | HG52.357F | GGTGGCGGCGGTGAGCAT (SEQ.ID.NO.:12) |
| | HG52.526R | AAGCAGCACCCCGAATACCA (SEQ.ID.NO.:13) |
| | HG52.F91 | TCCCGGCTCTGAGGCAC (SEQ.ID.NO.:14) |
| 30 | HG52.R242 | TGCAGCGTCGCGTGTGCC (SEQ.ID.NO.:15) |
| | PBS.543R | GGGGATGTGCTGCAAGGCGA (SEQ.ID.NO.:16) |
| | PBS.578R | CCAGGGTTTTCCAGTCACGAC (SEQ.ID.NO.:17) |
| | PBS.873F | CCCAGGCTTTACTTTATGCTTCC (SEQ.ID.NO.:18) |
| | PBS.838F | TTGTGTGGAATTGTGAGCGGATAAC (SEQ.ID.NO.:19) |

35

EXAMPLE 2

Tissue distribution of HG52 RNA transcripts

The data shown in Figure 4 were obtained by the use of human multi-tissue Northern blots purchased from Clontech (Palo Alto, CA). Hybridizations were
5 carried out as suggested by Clontech using the entire coding region of HG52 as probe.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art
10 from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED:

1. A recombinant DNA molecule encoding a polypeptide having the amino acid sequence SEQ.ID.NO.:2.

2. The DNA molecule of claim 1 comprising a nucleotide sequence selected from the group consisting of SEQ.ID.NO.:1 and positions 23-1,099 of SEQ.ID.NO.:1.

3. A DNA molecule that hybridizes under stringent conditions to the DNA of claim 1.

4. An expression vector comprising the DNA of claim 1.

5. A recombinant host cell comprising the DNA of claim 1.

6. An isolated HG52 protein having the amino acid sequence SEQ.ID.NO.:2.

7. The isolated HG52 protein of claim 6 that is substantially free from other proteins.

8. The HG52 protein of claim 6 containing a single amino acid substitution.

9. The HG52 protein of claim 6 containing two or more amino acid substitutions where the substitutions are conservative and do not occur in positions where HG52 and the human thrombin receptor 1 share the same amino acid.

10. A method for determining whether a substance is a potential agonist or antagonist of HG52 comprising:

- (a) transfecting cells with an expression vector encoding HG52;
- (b) allowing the transfected cells to grow for a time sufficient to allow HG52 to be expressed;

(c) exposing the cells to a labeled ligand of HG52 in the presence and in the absence of the substance;

- (d) measuring the binding of the labeled ligand to HG52; where if the amount of binding of the labeled ligand is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of HG52;

where HG52 has an amino acid sequence of SEQ.ID.NO.:2.

11. A method for determining whether a substance is capable of binding to HG52 comprising:

(a) providing test cells by transfecting cells with an expression vector that directs the expression of HG52 in the cells;

(b) exposing the test cells to the substance;

(c) measuring the amount of binding of the substance to HG52;

- 15 (d) comparing the amount of binding of the substance to HG52 in the test cells with the amount of binding of the substance to control cells that have not been transfected with HG52;

wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to HG52;

- 20 where HG52 has an amino acid sequence of SEQ.ID.NO.:2.

12. A method for determining whether a substance is capable of binding to HG52 comprising:

- 25 (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG52 in the cells;

(b) preparing membranes containing HG52 from the test cells and exposing the membranes to a ligand of HG52 under conditions such that the ligand binds to the HG52 in the membranes;

- 30 (c) subsequently or concurrently to step (b), exposing the membranes from the test cells to a substance;

(d) measuring the amount of binding of the ligand to the HG52 in the membranes in the presence and the absence of the substance;

(e) comparing the amount of binding of the ligand to HG52 in the membranes in the presence and the absence of the substance where a decrease in the

amount of binding of the ligand to HG52 in the membranes in the presence of the substance indicates that the substance is capable of binding to HG52;

where HG52 has an amino acid sequence of SEQ.ID.NO.:2.

- 5 13. A method for determining whether a substance is capable of binding to HG52 comprising:
- (a) providing test cells by transfecting cells with an expression vector that directs the expression of HG52 in the cells;
- (b) preparing membranes containing HG52 from the test cells and
- 10 exposing the membranes from the test cells to the substance;
- (c) measuring the amount of binding of the substance to the HG52 in the membranes from the test cells;
- (d) comparing the amount of binding of the substance to HG52 in the membranes from the test cells with the amount of binding of the substance to
- 15 membranes from control cells that have not been transfected with HG52;
- where HG52 has an amino acid sequence of SEQ.ID.NO.:2;
- where if the amount of binding of the substance to HG52 in the membranes from the test cells is greater than the amount of binding of the substance to the membranes from the control cells, then the substance is capable of binding to
- 20 HG52.
14. A method of identifying antagonists of HG52 comprising:
- (a) providing cells that expresses a chimeric HG52 protein fused at its C-terminus to a promiscuous G-protein;
- 25 (b) exposing the cells to an agonist of HG52;
- (c) subsequently or concurrently to step (b), exposing the cells to a substance that is a suspected antagonist of HG52;
- (d) measuring the level of inositol phosphates in the cells;
- where a decrease in the level of inositol phosphates in the cells in the
- 30 presence of the substance as compared to the level of inositol phosphates in the cells in the absence of the substance indicates that the substance is an antagonist of HG52.
15. A method of identifying agonists of HG52 comprising:
- (a) providing cells that expresses both HG52 and a promiscuous
- 35 G-protein;

(b) exposing the cells to a substance that is a suspected agonist of HG52;

- (c) measuring the level of inositol phosphates in the cells;
 where an increase in the level of inositol phosphates in the cells as
 5 compared to the level of inositol phosphates in the cells in the absence of the
 suspected agonist indicates that the substance is an agonist of HG52;
 where HG52 has an amino acid sequence of SEQ.ID.NO.:2.

16. A method of identifying antagonists of HG52 comprising:

- (a) providing cells that expresses both HG52 and a promiscuous
 10 G-protein;
 (b) exposing the cells to a substance that is an agonist of HG52;
 (c) subsequently or concurrently to step (b), exposing the cells to a
 substance that is a suspected antagonist of HG52;
 15 (d) measuring the level of inositol phosphates in the cells;
 where a decrease in the level of inositol phosphates in the cells in the
 presence of the suspected antagonist as compared to the level of inositol phosphates
 in the cells in the absence of the suspected antagonist indicates that the substance is
 an antagonist of HG52;
 20 where HG52 has an amino acid sequence of SEQ.ID.NO.:2.

17. An antibody that binds specifically to HG52 where HG52 has
 an amino acid sequence of SEQ.ID.NO.:2.

1/6

1 CCCGGGCGAG GACCCCTCCA GGATGCAGGT CCCGAACAGC ACCGGCCCCG
51 ACAACGCGAC GCTGCAGATG CTGCGGAACC CGGCGATCGC GGTGGCCCTG
101 CCGTGGTGT ACTCGCTGGT GGC GGCGGTG AGCATCCCGG GCAACCTCTT
151 CTCTCTGTGG GTGCTGTGCC GGC GCATGGG GCCAGATCC CCGTCGGTCA
201 TCTTCATGAT CAACCTGAGC GTCACGGACC TGATGCTGGC CAGCGTGTTG
251 CCTTTCAAA TCTACTACCA TTGCAACCGC CACCACTGGG TATTCGGGGT
301 GCTGCTTTGC AACGTGGTGA CCGTGGCCTT TTACGCAAAAC ATGTATTCCA
351 GCATCCTCAC CATGACCTGT ATCAGCGTGG AGCGCTTCCT GGGGGTCTCG
401 TACCCGCTCA GCTCCAAGCG CTGGCGCCGC CGTCGTTACG CGGTGGCCGC
451 GTGTGCAGGG ACCTGGCTGC TGCTCCTGAC CGCCCTGTCC CCGCTGGCGC
501 GCACCGATCT CACCTACCCG GTGCACGCCC TGGGCATCAT CACCTGCTTC
551 GACGTCCTCA AGTGGACGAT GCTCCCCAGC GTGGCCATGT GGGCCGTGTT
601 CCTCTTCAAC ATCTTCATCC TGCTGTTCTT CATCCCGTTC GTGATCACCG
651 TGGCTTGTTA CACGGCCACC ATCCTCAAGC TGTTCGCGAC GGAGGAGGCG
701 CACGGCCGGG AGCAGCGGAG GCGCGCGGTG GGCCTGGCGG CGGTGGTCTT
751 GCTGGCCTTT GTCACCTGCT TCGCCCCAA CAACTTCGTG CTCCTGGCGC
801 ACATCGTGAG CCGCCTGTTT TACGGCAAGA GCTACTACCA CGTGTACAAG
851 CTCACGCTGT GTCTCAGCTG CCTCAACAAC TGTCTGGACC CGTTTGTTTA
901 TTACTTTGCG TCCCGGAAT TCCAGCTGCG CCTGCGGGAA TATTTGGGCT
951 GCCGCCGGGT GCCCAGAGAC ACCCTGGACA CGCGCCGCGA GAGCCTCTTC
1001 TCCGCCAGGA CCACGTCCGT GCGCTCCGAG GCCGGTGCGC ACCCTGAAGG
1051 GATGGAGGGA GCCACGAGG CCGGCCTCCA GAGGCAGGAG AGTGTGTTCT
1101 GAGTCCGGG GGC GCAGCTT GGAGAGCCGG GGGCGCAGCT TGGAGATCC
1151 GGGGCGCATG GAGAGGCCAC GGTGCCAGAG GTTCAGGGAG AA

FIG. 1

FIG. 2. Predicted polypeptide sequence of HG52.

1 MQVPNSTGPD NATLQMLRNP AIAVALPVVY SLVAASVIG NLFSLWVLCR
51 RMGPRSPSVI FMINLSVTDL MLASVLPFQI YYHCNRHHWV FGVLLCNVVT
101 VAFYANMYSS ILTMTFCISVE RFLGVLYPLS SKRWRRRRYA VAACAGTWLL
151 LLTALSPLAR TDLTYPVHAL GIITCFDVLK WTMGPSVAMW AVFLFTIFIL
201 LFLIPFVITV ACYTATILKL LRTEEAHGRE QRRRAVGLAA VVLLAFVTCF
251 APNNFVLLAH IVSRLFYGKS YYHVYKLTLC LSCINNCDDP FVYFASREF
301 QLRLREYLGC RRVPRDTLDT RRESLFSART TSVRSEAGAH PEGMEGATRP
351 GLQRQESVF

3/6

10 30 50
CCGGGGCAGGACCCCTCCAGGATGCAGGTCCTCCGAACAGCACCCGGCCCGGACAACGCGACG
MetGlnValProAsnSerThrGlyProAspAsnAlaThr

70 90 110
CTGCAGATGCTGCGGAACCCGGCGATCGCGGTGGCCCTGCCGTGGTGTA CTCTCGTGGTG
LeuGlnMetLeuArgAsnProAlaIleAlaValAlaLeuProValValTyrSerLeuVal

130 150 170
GCGGCGGTGAGCATCCCGGGCAACCTCTTCTCTCTGTGGGTGTGTGCGGCGCATGGGG
AlaAlaValSerIleProGlyAsnLeuPheSerLeuTrpValLeuCysArgArgMetGly

190 210 230
CCCAGATCCCCGTGGTGCATCTTCATGATCAACCTGAGCGTCACGGACCTGATGCTGGCC
ProArgSerProSerValIlePheMetIleAsnLeuSerValThrAspLeuMetLeuAla

250 270 290
AGCGTGTGGCTTTCCAAATCTACTACCATTGCAACCGCCACCACTGGGTATTCGGGGTG
SerValLeuProPheGlnIleTyrTyrHisCysAsnArgHisHisTrpValPheGlyVal

310 330 350
CTGCTTTGCAACGTGGTGACCGTGGCCTTTTACGCAACATGTATTCCAGCATCCTCACC
LeuLeuCysAsnValValThrValAlaPheTyrAlaAsnMetTyrSerSerIleLeuThr

370 390 410
ATGACCTGTATCAGCGTGGAGCGCTTCTGGGGTCTGTACCCGCTCAGTCCAAGCGC
MetThrCysIleSerValGluArgPheLeuGlyValLeuTyrProLeuSerSerLysArg

430 450 470
TGGCGCGCCGCTCGTTACGCGGTGGCGCGTGTGCAGGGACCTGGCTGCTGCTCCTGACC
TrpArgArgArgArgTyrAlaValAlaAlaCysAlaGlyThrTrpLeuLeuLeuLeuThr

490 510 530
GCCCTGTCTCCCGCTGGCGCGCACCGATCTCACCTACCCGGTGCACGCCCTGGGCATCATC
AlaLeuSerProLeuAlaArgThrAspLeuThrTyrProValHisAlaLeuGlyIleIle

550 570 590
ACCTGCTTCGACGTCCTCAAGTGGACGATGCTCCCCAGCGTGGCCATGTGGGCGGTGTTT
ThrCysPheAspValLeuLysTrpThrMetLeuProSerValAlaMetTrpAlaValPhe

Fig.3A

4/6

610 630 650
 CTCTTCACCATCTTCATCTGCTGTTCTCATCCCGTTTCGTGATCACCGTGGCTTGTAC
 LeuPheThrIlePheIleLeuLeuPheLeuIleProPheValIleThrValAlaCysTyr

670 690 710
 ACGGCCACCATCCTCAAGCTGTTGCGCACGGAGGAGCGCACGGCCGGGAGCAGCGGAGG
 ThrAlaThrIleLeuLysLeuLeuArgThrGluGluAlaHisGlyArgGluGlnArgArg

730 750 770
 CGCGCGGTGGGCTGGCCGCGGTGGTCTTGCTGGCCTTTGTACCTGCTTCGCCCCCAAC
 ArgAlaValGlyLeuAlaAlaValValLeuLeuAlaPheValThrCysPheAlaProAsn

790 810 830
 AACTTCGTGCTCCTGGCGCACATCGTGAGCCGCTGTTCTACGCCAAGAGCTACTACCAC
 AsnPheValLeuLeuAlaHisIleValSerArgLeuPheTyrGlyLysSerTyrTyrHis

850 870 890
 GTGTACAAGCTCACGCTGTGTCTCAGCTGCCTCAACAACTGTCTGGACCCGTTTGTATTAT
 ValTyrLysLeuThrLeuCysLeuSerCysLeuAsnAsnCysLeuAspProPheValTyr

910 930 950
 TACTTTGCGTCCCGGGAATTCAGCTGCGCTGCGGGAATATTGGGCTGCCGCGGGGTG
 TyrPheAlaSerArgGluPheGlnLeuArgLeuArgGluTyrLeuGlyCysArgArgVal

970 990 1010
 CCCAGAGACACCCTGGACACGCGCCGCGAGAGCCTCTTCTCCGCCAGGACCAGTCCGTG
 ProArgAspThrLeuAspThrArgArgGluSerLeuPheSerAlaArgThrThrSerVal

1030 1050 1070
 CGCTCCGAGGCCGGTGCGCACCTGAAGGGATGGAGGGAGCCACCAGGCCCGGCTCCAG
 ArgSerGluAlaGlyAlaHisProGluGlyMetGluGlyAlaThrArgProGlyLeuGln

1090 1110 1130
 AGGCAGGAGAGTGTGTTCTGAGTCCCGGGGGCGCAGCTTGGAGAGCCGGGGGCGCAGCTT
 ArgGlnGluSerValPhe

1150 1170 1190
 GGAGATCCAGGGGCGCATGGAGAGGCCACGGTGCCAGAGTTCAGGGAGAA

Fig.3B

5/6

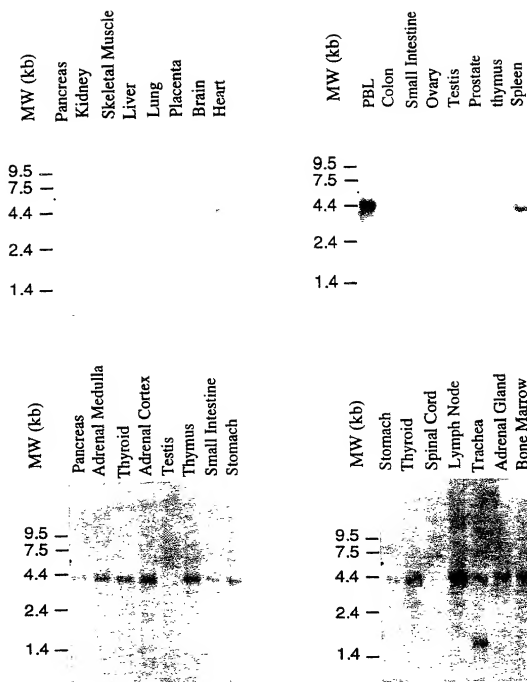


FIG.4

6/6

		10	20	30	
HG52 . pep		MQVPNSTGPDNATLQMLRNPAlAVALPVVYS			
		:	: :	: :	::: : :
hTHRR	EPFWEDEEKNESGLTEYRLVSI	INKSSPLQKQLPAFISE	DASG--	YLTSSWLT	LFVPSVYT
	60	70	80	90	100 110
	40	50	60	70	80 90
HG52 . pep	LVAANVIPGNLFSWLCCRMPGRSPSVIFMINLSVTDLMLASVLPFQIYYHCNRHHWVF				
	: :	:::	: :	::: : :	::: : :
hTHRR	GVFVVSPLPNIMAI	VVFILKMKVKKPAVVYMLHLATADVL	FVSVLPFKIS	YFSGSDWQF	
	120	130	140	150	160 170
	100	110	120	130	140 150
HG52 . pep	GVLLCNVTVAFYANMYSSILTMTCSIVERFLGVLYPLSSKRWRRRYAVAACAGTWLLL				
			: :	: :	: :
hTHRR	GSELCRFVTA	AFYCNMYASILLMTVISIDRF	LA	VVPMQSLSWRTLGRASFTCLAIWALA	
	180	190	200	210	220 230
	160	170	180	190	200 210
HG52 . pep	LTALSPLARTDITYPVHALGIITCFDVLKWTMLPSVAMWAVFLFTIF-ILLFLIPFVITV				
	::: :	: :	: :	: :	: :
hTHRR	IAGVVPLVLKEQTIQVPGLNITTCHDVLNETLLEG--	YYAYFSAFSAVFFVPLIIST			
	240	250	260	270	280
	220	230	240	250	260
HG52 . pep	ACYTATILKLLRTEEAHGREQRRRAVGLAAVLLAFVTCFAPNNFVLLAH--IVSRLFYG				
	: :	: :	: :	: :	: :
hTHRR	VCYV-SIIRCLSSSAVANRSKKSRAFLSA	AVFCIFIICFGPTNVL	LI	IAHYSFLSHTSTT	
	290	300	310	320	330 340
	270	280	290	300	310 320
HG52 . pep	KSYHHVYKLTCLSLNCLDPFVYVFASREFQLRLREYLGCRRVPRDLDTRRESLFS				
	: :	: :	: :	: :	: :
hTHRR	EAAYFAYLLCVCVSSISSCIDPLIYYASSECQRYVYSILCKESSDPSSYNSSGQLMAS				
	350	360	370	380	390 400
	330	340	350	360	
HG52 . pep	RTTSVRSEAGAHPEGMEGATRPGLRQESVF				
hTHRR	KMDTCSSNLNNSIYKLLT				
	410	420			

FIG.5

**DECLARATION AND
POWER OF ATTORNEY
FOR UTILITY OR DESIGN
PATENT APPLICATION
(37 CFR 1.63)**

☐ Declaration Submitted with Initial Filing ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required) OR

Attorney Docket Number 20330P

First Named Inventor Liu, et al.

COMPLETE IF KNOWN

Application Number 09/806,087

Filing Date March 26, 2001

Group Art Unit

Examiner Name

**As a below named inventor, I hereby declare that:**

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

G PROTEIN-COUPLED RECEPTOR RESEMBLING THE THROMBIN RECEPTOR

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 03/26/2001 as United States Application Number or PCT International

Application Number 09/806,087 and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Attorney Docket Number	Priority Claimed?	
				YES	NO
PCT/US99/22634	PCT	09/29/1999	20330-PCT	<input checked="" type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>
				<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	Attorney Docket Number
60/102,958	10/02/1998	20330PV

DECLARATION AND POWER OF ATTORNEY for Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information known to me to be material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Application Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)
60/102,958	10/02/1998	20330PV
PCT/US99/22634	09/29/1999	20330-PCT

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint, respectively and individually, as my attorneys or agents with full power of substitution and revocation, the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

☐ Customer Number

OR

☒ Registered practitioner(s) name/registration number listed below

Place Customer Number
Bar Code Label here

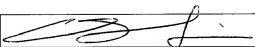
Name	Registration Number	Name	Registration Number
Joseph A. Coppola	38,413	Jack L. Tribble	32,633

Direct all correspondence to: ☒ Customer Number or Bar Code Label

000210

Name	Joseph A. Coppola		
Address	Merck & Co., Inc. - Patent Department		
Address	P.O. Box 2000, RY60-30		
City	Rahway	State	NJ
ZIP	07065-0907		
Country	USA	Telephone	(732)594-6734
Fax	(732)594-4720		

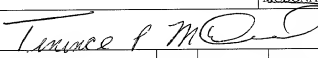
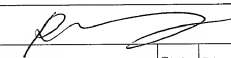
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:	<input type="checkbox"/> A petition has been filed for this unsigned inventor		
Given Name (first and middle (if any))	Family Name or Surname		
QINGYUN	LIU		
Inventor's Signature			Date
Residence: City	North Wales	State	PA
Country	US		Citizenship
Post Office Address	Merck & Co., Inc., P.O. Box 2000		
City	Rahway	State	NJ
ZIP	07065-0907		

☒ Additional inventors are being named on the 1 supplemental Additional Inventors(s) sheet(s) PTO/SB/02A attached hereto.

DECLARATION AND POWER OF ATTORNEY

ADDITIONAL INVENTOR(S)
Supplemental Sheet

Name of Additional Joint Inventor, if any:		A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])		Family Name or Surname					
TERRENCE P		MCDONALD					
Inventor's Signature						Date	June 04, 2001
Residence: City	Langhorne	State	PA	Country	US	Citizenship	US
Post Office Address	Merck & Co., Inc., P.O. Box 2000 US						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])		Family Name or Surname					
RUIPING		WANG					
Inventor's Signature						Date	June 4, 2001
Residence: City	Maple Glen	State	PA	Country	US	Citizenship	CN
Post Office Address	Merck & Co., Inc., P.O. Box 2000 US						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])		Family Name or Surname					
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		
Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle [if any])		Family Name or Surname					
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address	Merck & Co., Inc., P.O. Box 2000						
City	Rahway	State	NJ	ZIP	07065-0907		